

WHAT IS CLAIMED IS:

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- 1 1. An isolated DNA molecule selected from the group consisting of:
    - 2 A. the DNA sequence of FIGURE 1 (SEQ ID NO:1);
    - 3 B. the DNA sequence of FIGURE 2 (SEQ ID NO:3);
    - 4 C. the DNA sequence of FIGURE 20A (SEQ ID NO:22);
    - 5 D. DNA sequences that hybridize to any of the foregoing DNA
    - 6 sequences under standard hybridization conditions;
    - 7 E. DNA sequences that code on expression for an amino acid sequence
    - 8 encoded by any of the foregoing DNA sequences;
    - 9 F. degenerate variants thereof;
    - 10 G. alleles thereof; and
    - 11 H. hybridizable fragments thereof.
  - 1 2. An isolated nucleic acid molecule, which nucleic acid molecule encodes an  
2 ob polypeptide, which polypeptide is characterized by having about 145 to about  
3 167 amino acid residues, being expressed predominantly by adipocytes, and being  
4 capable of inducing a reduction of body weight in an animal.
  - 1 3. The isolated nucleic acid of Claim 2, wherein the ob polypeptide has an  
2 amino acid sequence selected from the group consisting of the sequence depicted  
3 in Figure 1 (SEQ ID NO:2), Figure 1 from amino acid number 22 to amino acid  
4 number 167, Figure 3 (SEQ ID NO:4), Figure 3 from amino acid number 22 to  
5 amino acid number 167, Figure 5 (SEQ ID NO:5), Figure 5 from amino acid  
6 number 22 to amino acid number 166, Figure 6 (SEQ ID NO:6), and Figure 6  
7 from amino acid number 22 to amino acid number 166.
  - 1 4. The nucleic acid molecule of Claim 2 selected from the group consisting of  
2 DNA and RNA.

- 1 5. The nucleic acid molecule of Claim 2, which has a sequence as shown in  
2 Figure 1 (SEQ ID NO:1) from nucleotide number 46 to nucleotide number 550.
- 1 6. The nucleic acid molecule of Claim 2, which has a sequence as shown in  
2 Figure 2 (SEQ ID NO:2) from nucleotide number 46 to nucleotide number 550.
- 1 7. The nucleic acid molecule of Claim 1 which is detectably labeled.
- 1 8. A cloning vector, which comprises the DNA molecule of Claim 1.
- 1 9. An expression vector, which comprises the nucleic acid molecule of Claim  
2 2, operatively associated with an expression control sequence.
- 1 10. The expression vector of Claim 9, wherein said expression control  
2 sequence is selected from the group consisting of the cytomegalovirus hCMV  
3 immediate early gene, the early or late promoters of SV40 or adenovirus, the lac  
4 system, the trp system, the TAC system, the TRC system, the major operator and  
5 promoter regions of phage  $\lambda$ , the control regions of fd coat protein, the promoter  
6 for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the  
7 promoters of the yeast  $\alpha$ -mating factors.
- 1 11. A probe capable of screening for a nucleic acid encoding an ob polypeptide  
2 in alternate species, which probe is a labeled DNA molecule of Claim 1.
- 1 12. A unicellular host transfected with a cloning vector of Claim 8.
- 1 13. A unicellular host transfected with an expression vector of Claim 9.
- 1 14. The unicellular host of Claim 13 wherein the unicellular host is selected  
2 from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Pichia*

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3 yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells,  
4 plant cells, insect cells, and human cells in tissue culture.

1 15. An oligonucleotide primer for amplifying human genomic DNA encoding  
2 an ob polypeptide.

1 16. The oligonucleotide of Claim 15, which is selected from the group  
2 consisting of

3 HOB 1gF 5'-CCCAAGAAGCCCATCCTG-3' (SEQ ID NO:26)  
4 HOB 1gR 5'-GACTATCTGGGTCCAGTGCC-3' (SEQ ID NO:27)  
5 HOB 2gF 5'-CCACATGCTGAGCACTTGTT-3' (SEQ ID NO:28)  
6 HOB 2gR 5'-CTTCAATCCTGGAGATACCTGG-3' (SEQ ID NO:29).

1 17. An ob polypeptide, which polypeptide is encoded by the DNA molecule of  
2 Claim 1.

1 18. An ob polypeptide, which polypeptide is characterized by having about 145  
2 to about 167 amino acid residues, being expressed predominantly by adipocytes,  
3 and being capable of inducing a reduction of body weight in an animal.

1 19. The ob polypeptide of Claim 18 which has the amino acid sequence shown  
2 in Figure 1 (SEQ ID NO:2) or Figure 5 (SEQ ID NO:5).

1 20. The ob polypeptide of Claim 19 which has the amino acid sequence shown  
2 in Figure 3 (SEQ ID NO:4) or Figure 6 (SEQ ID NO:6).

1 21. An immunogenic fragment of an ob polypeptide, which polypeptide is  
2 characterized by having about 160 amino acid residues, being expressed  
3 predominantly by adipocytes, and being capable of inducing a reduction of body  
4 weight in an animal.

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- 1 22. The immunogenic fragment of an ob polypeptide of Claim 21, which is  
2 selected from the group consisting of  
3 Val-Pro-Ile-Gln-Lys-Val-Gln-Asp-Asp-Thr-Lys-Thr-Leu-Ile-Lys-Thr (SEQ  
4 ID NO:18);  
5 Leu-His-Pro-Ile-Leu-Ser-Leu-Ser-Lys-Met-Asp-Gln-Thr-Leu-Ala (SEQ ID  
6 NO:19);  
7 Ser-Lys-Ser-Cys-Ser-Leu-Pro-Gln-Thr-Ser-Gly-Leu-Gln-Lys-Pro-Glu-Ser-  
8 Leu-Asp (SEQ ID NO:20); and  
9 Ser-Arg-Leu-Gln-Gly-Ser-Leu-Gln-Asp-Ile-Leu-Gln-Gln-Leu-Asp-Val-Ser-  
10 Pro-Glu-Cys (SEQ ID NO:21).
- 1 23. A method for preparing an ob polypeptide comprising:  
2 A. culturing a unicellular host of Claim 12 or 13 under conditions that  
3 provide for expression of the ob polypeptide; and  
4 B. recovering the expressed ob polypeptide.
- 5 24. The method according to Claim 23 wherein the host cell is a bacterium.
- 1 25. The method according to Claim 23, wherein the host cell is a yeast.
- 1 26. The method according to Claim 23, further comprising:  
2 C. chromatographing the polypeptide on a Ni-chelation column; and  
3 D. purifying the polypeptide by gel filtration.
- 1 27. The method according to Claim 26, further comprising after step C and  
2 before step D chromatographing the ob polypeptide on a strong cation exchanger  
3 column.
- 1 28. An antibody to the ob polypeptide of Claim 17.
- 1 29. An antibody to the ob polypeptide of Claim 18.

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- 1 30. A method for preparing an antibody to an ob polypeptide, comprising:  
2 A. conjugating the immunogenic fragment of an ob polypeptide of  
3 Claim 19 to a carrier protein;  
4 B. immunizing a host animal with the ob polypeptide fragment-carrier  
5 protein conjugate of step A admixed with an adjuvant; and  
6 C. obtaining antibody from the immunized host animal.

- 1 31. An antibody to an ob polypeptide prepared according to a method  
2 comprising:  
3 A. conjugating an immunogenic fragment of an ob polypeptide of  
4 Claim 19 to a carrier protein;  
5 B. immunizing a host animal with the ob polypeptide fragment-carrier  
6 protein conjugate of step A admixed with an adjuvant; and  
7 C. obtaining antibody from the immunized host animal.

- 1 32. The antibody of Claim 28, 29, or 31 comprising a polyclonal antibody.

- 1 33. The antibody of Claim 28, 29, or 30 comprising a monoclonal antibody.

- 1 34. An immortal cell line that produces a monoclonal antibody according to  
2 Claim 33. *B*

- 1 35. The antibody of Claim 28, 29, or 31 labeled with a detectable label.

- 1 36. The antibody of Claim 35 wherein the label is selected from the group  
2 consisting of enzymes, chemicals which fluoresce, and radioactive elements.

- 1 37. A method for measuring the presence of an ob polypeptide in a sample,  
2 comprising:  
3 A. contacting a sample suspected of containing an ob polypeptide with  
4 an antibody that binds to the ob polypeptide under conditions which allow for the

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9 ob polypeptide in the sample.

1 38. The method of Claim 37 in which the antibody is bound to a solid phase  
2 support.

1 39. The method of Claim 38 which further comprises contacting the sample  
2 with a labelled ob polypeptide step (A), and removing unbound substances prior to  
3 step (B), and in which the formation of reaction complexes in the sample is  
4 detected by observing a decrease in the amount of labelled ob polypeptide in the  
5 sample.

1 40. The method of Claim 38 which further comprises contacting the sample  
2 with a labelled antibody in step (A), which labelled antibody is an anti-ob  
3 polypeptide antibody, and removing unbound substances prior to step (B), and in  
4 which the formation of reaction complexes in the sample is detected by observing  
5 an increase in the amount of labelled antibody in the sample.

1 41. The method of Claim 37 in which an ob polypeptide is bound to a solid  
2 phase support.

1 42. The method of Claim 41 which further comprises contacting the sample  
2 with an ob polypeptide in step (A), and removing unbound substances prior to step  
3 (B), and in which the antibody is labelled and the formation of reaction complexes  
4 in the sample is detected by observing a decrease in the amount of labelled  
5 antibody.

1 46. The method according to Claim 44 or 45, wherein the disease associated  
2 with elevated levels of ob polypeptide is selected from the group consisting of  
3 AIDS, cachexia, cancer, and anorexia nervosa.

1 48. A test kit for measuring the presence or amount of ~~ob~~ polypeptide in a  
2 sample, comprising:

1 49. A method for changing the body weight of a mammal comprising inhibiting  
2 the expression of an ob polypeptide encoded by a nucleic acid of Claim 2.

1 50. The method according to Claim 49 comprising expressing an antisense  
2 nucleic acid molecule hybridizable to a nucleic acid that expresses the ob  
3 polypeptide, expressing a ribozyme that cleaves a nucleic acid that expresses the  
4 ob polypeptide, administering an antisense nucleic acid molecule hybridizable to a  
5 nucleic acid that expresses the ob polypeptide, and administering a ribozyme that  
6 cleaves a nucleic acid that expresses the ob polypeptide.

1 51. A pharmaceutical composition for reducing body weight of an animal  
2 comprising the ob polypeptide of Claim 17 and a pharmaceutically acceptable  
3 carrier.

1 52. A pharmaceutical composition for reducing body weight of an animal  
2 comprising the ob polypeptide of Claim 18 and a pharmaceutically acceptable  
3 carrier.



1 53. A method for reducing the body weight of an animal comprising  
2 administering an amount of a pharmaceutical composition of Claim 52 effective to  
3 reduce the body weight of an animal to an animal believed to be in need of  
4 decreased body weight.

1 54. The method according to Claim 53 wherein the animal is a human, and the  
2 ob polypeptide is human ob polypeptide.

1 55. A method for reducing the body weight of a mammal comprising increasing  
2 the expression of a protein encoded by the nucleic acid of Claim 2.

1 56. A pharmaceutical composition for increasing the body weight of an animal  
2 comprising an antagonist of an ob polypeptide.

1 57. The pharmaceutical composition of Claim 56, wherein the antagonist is  
2 selected from the group consisting of an antibody that binds to and neutralizes the  
3 activity of ob polypeptide, a fragment of the ob polypeptide that binds to but does  
4 not activate the ob receptor, and a small molecule antagonist of the ob  
5 polypeptide.

1 58. A method for increasing the body weight of an animal comprising  
2 administering an amount of the pharmaceutical composition of Claim 56 effective  
3 to cause an increase in body weight to an animal believed to be in need of  
4 increased body weight.

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